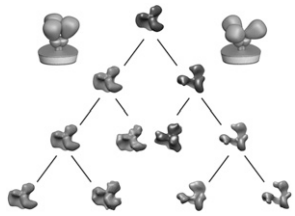
[1]NIH-NCI, Bethessda, MD, USA, [2]Office of High Performance Computing and Communications, NIH-NLM, Bethessda, MD, USA, [3]Department of Electrical and Computer Engineering - University of Minnesota, Minneapolis, MN, USA.

We have previously used cryo-electron tomography combined with missing-wedge corrected, sub-volume averaging and classification to obtain 3D structures of macromolecular assemblies in cases where a single dominant species was present, and applied these methods to the analysis of a variety of trimeric HIV-1 and SIV envelope glycoproteins (Env). Here, we extend these studies by presenting a method for determining the distribution of conformational states found in a specimen and validate these procedures by successfully separating and reconstructing distinct 3D structures for unliganded and antibody-liganded as well as open and closed conformations of Env present simultaneously in mixtures. We show that identifying and removing spikes with the lowest signal-to-noise ratios improves the overall accuracy of alignment between individual Env sub-volumes, and that alignment accuracy, in turn, determines the success of image classification in assessing conformational heterogeneity in heterogeneous mixtures. This development extends the sub-tomogram averaging capabilities to heterogeneous samples. Furthermore, it turns cryo-electron tomography into a powerful analytical tool that can directly determine the relative amount of the different conformations found in the specimen.



1799-Pos Board B691

Structural Studies of Dynamin-Related Protein 1 (DRP1) Provide Mechanistic Insight into Mitochondrial Fission.

Frances Alvarez, Louie Zhou, **Jason A. Mears**.
Case Western Reserve University, Cleveland, OH, USA.

Dynamin-related protein 1 (Drp1) belongs to a family of large GTPase proteins that regulate membrane dynamics and morphology. Drp1 localizes to mitochondrial constriction sites *in vivo* to facilitate outer membrane fission, and mutations that inhibit its activity lead to hyper-fused mitochondria *in vivo*. Direct inhibition of Drp1 protects against cell death by limiting increased mitochondrial fission associated with apoptosis. Using cryo-electron microscopy (cryo-EM), previous studies of the yeast homolog of Drp1, Dnm1, were used to determine its structural properties. Dnm1 was shown to form large (>100 nm in diameter) helical oligomers that constrict upon GTP hydrolysis to generate a contractile force on the underlying membrane. Similar methods are now being used to gain mechanistic insight into the mammalian mitochondrial fission complex. Several similarities and differences have been found between the yeast and mammalian systems. In solution, Drp1 forms stable tetramers, which represent the pre-assembled state of Drp1. The size of this complex (~330 kDa) provides a suitable target for 3D image reconstruction. Additional interactions with GTP analogs and/or synthetic liposomes promote Drp1 self-assembly into extended helical oligomers. The 3D structures of these helices will be determined to elucidate interactions that mediate Drp1 self-assembly. The effects of GTP hydrolysis on the Drp1 helical oligomers are also being studied to determine how Drp1 promotes outer mitochondrial membrane fission. Future studies will examine interactions between Drp1 and partner proteins in the mitochondrial fission complex.

1800-Pos Board B692

Electron Microscopy Structure of Dimeric LRRK2 Reveals a Structural and Regulatory Role of the COR Domain

Yacob Gómez Llorente[1], Fabiana Renzi[1], Xianting Li[1], William J. Rice[2], Jose Chavez[1], Nina Pan[1], James Powell[1], Zhenyu Yue[1], Iban Ubarretxena-Belandia[1].
[1]Mount Sinai School of Medicine, New York, NY, USA, [2]New York Structural Biology Center, New York, NY, USA.

Missense mutations in leucine-rich repeat kinase 2 (LRRK2) constitute the most common genetic cause of Parkinson disease (PD). LRRK2 is a 286-kDa multidomain protein containing several protein-protein interaction domains, including armadillo, leucine-rich and ankyrin repeats, as well as a Ras Of Complex proteins GTPase (ROC) and a kinase joined by a C-terminal Of ROC (COR) domain. PD-linked mutations are clustered in the catalytic core of LRRK2 suggesting that altered GTPase and kinase activities may be implicated in pathogenesis. Biochemical experiments suggest LRRK2 kinase activity may be regulated by dimerization. Electron microscopy imaging and single-particle 3D reconstruction, at a resolution of 22 Å, reveal that LRRK2 purified from mouse brain forms elliptical homodimers with each monomer having a concave lune shape. Dimerization occurs via a single two-fold rotation axis, in which the two monomers interact via two main interfaces. Details in the electron microscopy map provide insight into the domain organization of LRRK2. Docking of a prokaryotic ROC-COR homologous structure suggests LRRK2 dimerization may be mediated primarily by the COR domain. Immunoprecipitation experiments confirm the predicted COR-COR interaction. Furthermore, competition experiments showed the COR domain inhibits LRRK2 kinase activity in vitro. Our data reveal the COR domain to play a critical role at the dimerization interface and in the regulation of LRRK2 kinase activity.

1801-Pos Board B693

Emdatabank: Unified Data Resource for 3DEM

Catherine Lawson[1], Ardan Patwardhan[2], Grigore D. Pintilie[3], Eduardo Sanz Garcia[2], Ingvar Lagerstedt[2], Matthew L. Baker[3], Raul Sala[1], Steven J. Ludtke[3], Helen M. Berman[1], Gerard Kleywegt[2], Wah Chiu[3].
[1]Rutgers University, Piscataway, NJ, USA, [2]European Bioinformatics Institute, Hinxton, United Kingdom, [3]Baylor College of Medicine, Houston, TX, USA.

3D cryo-electron microscopy (3DEM) is emerging as a powerful method for determining structures of large biological assemblies in solution and in the cell, enabling elucidation of complex biological interactions that are integral to understanding the inner workings of cellular machinery, and yielding novel insights into fundamental biological processes. Hundreds of 3DEM experiments are now reported in the literature each year and more than 1,500 structures are now available through EMDataBank. The project website, EMDataBank.org, serves as a ''one-stop shop'' resource for global deposition and retrieval of 3DEM map and model data, and facilitates use of cryo-EM structural data by the wider scientific community. We will describe improvements to deposition and representation of map and model data in the EMDB and PDB public archives that will be rolled out within the 3DEM module of the new wwPDB deposition & annotation tool. We will also provide an update on our current candidate methods for assessing reliability of 3DEM maps and map-derived models in collaboration with community scientists, with the goal of creating validation criteria that will permit independent assessment of 3DEM data by expert and non-expert scientists.

1802-Pos Board B694

Domain Organization of Membrane-Bound Factor VIII

Svetla Stoilova-McPhie[1], Gillian C. Lynch[2], Steven Ludtke[3], Bernard M. Pettitt[4].
[1]UTMB, Galveston, TX, USA, [2]Sealy Center for Structural Biology and Molecular Biophysics, UTMB, Galveston, TX, USA, [3]National Center for Molecular Imaging, Baylor College of Medicine, Houston, TX, USA, [4]2Sealy Center for Structural Biology and Molecular Biophysics, University of Texas Medical Branch, Galveston, TX, USA.

Factor VIII (FVIII) is the blood coagulation protein which when defective or deficient causes for hemophilia A, a severe hereditary bleeding disorder. Activated FVIII (FVIIIa) is the co-factor to the serine protease Factor IXa (FIXa) within the membrane-bound Tenase complex, responsible for amplifying its proteolytic activity more than 100,000 times, necessary for normal blood clotting. FVIII is composed of two non-covalently linked peptide chains: a light chain holding the membrane interaction sites and a heavy chain holding the main FIXa interaction sites. The interplay between the light and heavy chains in the membrane-bound state is critical for FVIII biological efficiency.

Here, we present our cryo-electron microscopy and structure analysis studies of human FVIII light chain (LC), as helically assembled onto negatively charged single lipid bilayer nanotubes (LNT). The resolved FVIII-LC membrane-bound structure at 20 Å, supports aspects of our previously proposed FVIII structure from membrane-bound two-dimensional (2D) crystals, such as only the C2 domain interacts directly with the membrane [Stoilova-McPhie 2002]. The light chain is oriented differently in the FVIII membrane-bound helical and 2D crystal structures based on electron microscopy data and the 3D structure solved by X-ray [Ngo 2008]. The flexibility of the FVIII-LC domain organization in different crystal packing (3D, 2D and helical) is essential to understand the FVIII membrane-bound organization and its significance for hemostasis.

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